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TITLE: Proteomic Analyses of Nipple Fluids for Early Detection of Breast Cancer

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13. ABSTRACT (Maximum 200 Words)

The purpose of our work is to combine the well-established method of breast nipple fluid collection with the new proteomics methodology for analyses of complex protein mixtures, in order to seek a better test for early breast cancer. The scope of our work is far-reaching, as our results could have a significant impact on the ability to detect occult breast cancers at earlier stages than is possible with current cancer detection methods.

Our progress to date has been:

1. to work out a collection method that is efficient and obtains a high-yield of nipple aspirate fluid;
2. to determine that resuspending the aspirate in a small amount of PBS allows for full recovery of the sample; and
3. to develop a systematic protocol for collection that is easily adaptable to other sites.

In summary, we have developed a workable protocol for nipple aspirate collection that produces consistent quantitative protein analysis. We are looking forward to proceeding with sample collection for aims one and two once we have received IRB approval from the Department of Defense.

14. SUBJECT TERMS
Early breast cancer detection, screening, proteomics, breast nipple fluid, women at high risk

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INTRODUCTION:

When breast cancers are diagnosed by self-examination, clinical examination and/or mammography, the tumor is usually comprised of millions of genetically-unstable cancer cells, including subclones differing in responses to therapy. The goal of our project is to find a new method to detect occult breast cancers at earlier stages than is possible with current cancer detection methods. To achieve our goals, we have combined the well-established method of breast nipple fluid collection with the new proteomics methodology for analyses of complex protein mixtures, in order to seek a better test for early breast cancer. We aim to collect breast nipple fluids from 200 women with yet-untreated breast cancer (cases), and 200 cancer-free controls and perform proteomic analyses in 100 cases and 100 controls to identify nipple fluid protein patterns associated with the presence of breast cancer. Specific proteins and patterns will be validated by repeating the proteomic studies on a second blinded series of fluids from another series of 100 cases and 100 controls, link laboratory results with clinical and diagnostic data, and score the sensitivity and specificity of the assay. If successful, the proteomics assay might permit earlier breast cancer diagnosis, and thereby reduce disease morbidity and mortality.

BODY:

Task 1. Subject recruitment and consent (years 1 and 2)

a. Modify our currently active IRB-approved protocol (93-020: Collection and Laboratory Study of Breast Nipple Fluid Using a Nipple Suction Cup) to include proteomic analyses among the laboratory assays. ***Our currently active IRB-approved protocol has been modified to include proteomic analysis among the laboratory assays. We have been working with the Dana-Farber IRB and the Department of Defense IRB to develop a protocol that meets the requirements of both institutions. We recently submitted a third revision of our protocol to the DOD IRB and are awaiting a response.***
b. Obtain consent from eligible subjects and their physicians to enroll and collect breast nipple fluid samples, and demographic and clinical data from 200 women with newly diagnosed breast cancer.
c. In like manner, consent as control subjects 200 cancer-free women examined at the mammography centers within Dana-Farber/Harvard Cancer Center.
(Under a different funding source and approved protocol, we have begun to collect nipple aspirate specimens to work out the logistics of the collection process and proteomic analysis.)

Task 2. Nipple fluid collection (years 1 and 2)

No work has been done on this task

a. Use our previously-developed methods to collect nipple fluids into capillary tubes from these 400 subjects and deliver the specimens to Dr. Miron's laboratory.
b. In the laboratory, dilute these viscous nipple fluid samples with 1X phosphate-buffered saline, accession specimens and store at -70°C.

Task 3. Biomarker discovery in nipple fluid (years 1 and 2)

No work has been done on this task

a. Establish the automated nipple fluid fractionation and chip binding procedure using our Tecan robotic workstation and IMAC3, WCX2, and H4 chip surfaces.
b. Perform proteomic analyses on 100 aliquots of nipple fluids from patients with breast cancer and 100 cancer-free women, using SELDI-TOF methodology using each of the chip surfaces in duplicate.
c. Analyze the data and obtain the biomarker set.

Task 4. Identification and validation of nipple fluid biomarkers for cancer (years 2 and 3)

No work has been done on this task

a. Identify protein patterns in the arrays that differentiate nipple fluids obtained from cancerous breasts as compared with tumor-free breasts.
b. Catalogue the protein markers present in nipple fluids from cancerous breasts that are absent from nipple fluids of cancer-free women.
c. To validate the preliminary findings from the first set of samples, a third party will code the second series of 100 nipple fluid samples from breast cancer patients and 100 samples from cancer-free women.
d. Repeat the SELDI-TOF analyses in a blinded manner on the 100 case samples and 100 controls.
e. Analyze data for the 200 blinded samples and score the source of the sample as a cancer-bearing breast, normal breast or indeterminate origin.
f. Break the sample codes and quantify the sensitivity and specificity of the assay in differentiating breast cancer cases from healthy controls.
g. Identify causes of false-positive and false-negatives.

Task 5. Dissemination of results and, if appropriate, organize multi-center studies (year 3)

No work has been done on this task

a. Prepare manuscript(s) for peer-review and publication.
b. If the proteomic analysis is shown to have high predictive value, develop a multi-center collaborative to re-confirm the results in a larger study.
c. Seek additional funding to further refine the assay, and submit a Traditional Research Proposal.

KEY RESEARCH ACCOMPLISHMENTS:

- We have worked out a collection method that is efficient and obtains a high-yield of nipple aspirate fluid.
- We have determined that resuspending the aspirate in a small amount of PBS allows for full recovery of the sample.
- We have developed systematic protocol for collection that is easily adaptable to other sites.

REPORTABLE OUTCOMES:

Not applicable

CONCLUSIONS:

To date, we have developed a workable protocol for nipple aspirate collection that produces consistent quantitative protein analysis. We are looking forward to proceeding with sample collection for aims one and two once we have received IRB approval from the Department of Defense.

REFERENCES:

Not applicable

APPENDICES:

Not applicable